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TOXICOLOGICAL AND RECALCITRANT PROPERTIES OF A PROPOSED PROPELL--ETC(U)
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TOXICOLOGICAL AND RECALCITRANT PROPERTIES OF A PROPOSED PROPELLANT INGREDIENT, TRIAMINOGUANIDINE NITRATE (TAGN)

III. TOXICITY TO DROSOPHILA MELANOGASTER

ENVIRONICS OFFICE

MARCH 1977

FINAL REPORT FOR PERIOD
APRIL 1976-MARCH 1977

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AIR FORCE ARMAMENT LABORATORY
AIR FORCE SYSTEMS COMMAND • UNITED STATES AIR FORCE
EGLIN AIR FORCE BASE, FLORIDA

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Triaminoguanidine nitrate	Gun propellant
TAGN	Toxicity
Drosophila melanogaster	Environmental effects

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The Air Force is considering the use of triaminoguanidine nitrate (TAGN) in future gun propellants, especially for high density projectiles. The effects of TAGN on Drosophila melanogaster were determined by administering the chemical via their growth medium. Toxicological and reproductive effects were determined at concentrations up to 4000 ppm TAGN. Increasing the concentration of TAGN to 1000 ppm in the medium resulted in almost all cessation of pupae and larvae production as well as an approximately 50 percent death of adult Drosophila.

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Item 20 concluded: Concentrations of 2000 ppm TAGN and above resulted in the death of almost all adult Drosophila. Concentrations of these levels are considered to be relatively high, however, for practical use. Therefore, TAGN appeared to be relatively non-toxic to Drosophila.

PREFACE

This technical report discusses a portion of the work performed at the Air Force Armament Laboratory, Armament Development and Test Center, Eglin Air Force Base, Florida, under Exploratory Development Project 5066 during the period April 1976 through February 1977.

The sources and manufacturers of materials and equipment used in this study are identified for reference only and do not constitute endorsement of the companies or products by the United States Air Force.

The contribution by Mr James C. Richardson in assisting in the statistical design and analyses of this study is gratefully acknowledged.

This technical report has been reviewed and is approved for publication.

This report has been reviewed by the Information Office (OI) and is releaseable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

FOR THE COMMANDER

Joe A. Farmer

JOE A. FARMER
Chief, Environics Office

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TABLE OF CONTENTS

LIST OF FIGURES

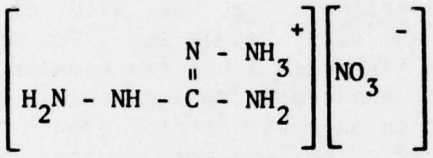
LIST OF TABLES

SECTION I

INTRODUCTION

The Air Force is considering the use of triaminoguanidine nitrate (TAGN) in future gun propellants, especially for high-density projectiles. Very little environmental or toxicological information is available for this compound. In anticipation of the requirement for environmental assessments as this new propellant material goes through development and testing and possibly into production, this project was initiated to provide a portion of the data required for the assessment.

TAGN is a white crystalline powder with a molecular weight of 167.139 (Reference 1).



No information about the toxicity of TAGN has been found in the literature since it is an infrequently used compound. Consequently, a research plan was initiated to determine the toxicological effects of this compound on microorganisms, fruit flies, and mammals. This report covers only the portion dealing with fruit flies (Drosophila melanogaster). Other data have already been included in previous technical reports (References 2, 3) or will be included in a future report.

Drosophila melanogaster has been used predominantly as an organism for genetic studies. However, because of its relatively short life cycle (10 days at 25°C), small size permitting ease of handling, relatively small space requirements, and its ease in breeding and reproduction which allows sufficiently large numbers of organisms to permit statistical accuracy, Drosophila has also been used frequently for toxicity studies (References 4, 5, 6, 7, 8).

This experiment using Drosophila as the test organism was conducted in two parts: Part I - Effects on Adult Flies, and Part II - Reproductive Effects.

SECTION II

METHODS AND MATERIALS

PART I - EFFECTS ON ADULT FLIES

On 21 April 1976, 40 250-ml sterile culture jars were prepared by adding 15 grams of Carolina Biological Supply Company Instant Drosophila Medium, Formula 4-24®, to each jar. Concentrations of 0 parts per million (ppm), 1000 ppm, 2000 ppm, 3000 ppm, and 4000 ppm of TAGN were prepared in distilled water and thoroughly dissolved. Fifty ml of each concentration were then added to each of eight jars. After the media solidified, a small amount of yeast was added. Each group of eight jars containing a given concentration of TAGN was then divided into replications 1 through 8.

Specimens of Drosophila melanogaster, var. wild, of less than 48 hours age were then anesthetized and separated by sex. Ten males were placed in each jar of replications 1 through 4 and ten females in each jar of replications 5 through 8 in each concentration group. After the flies revived, the jars were placed in an environmental growth chamber set at 23°C and a photoperiod of 17 hours. The jars were monitored for several hours to determine if transfer procedures resulted in any deaths. The number of dead flies were then recorded on days 1, 2, 5, 7, 9, and 12 after the experiment was initiated.

PART II - REPRODUCTIVE EFFECTS

On 23 June 1976, 40 250-ml sterile culture jars were prepared by adding 15 grams of Carolina Biological Supply Company Instant Drosophila Medium, Formula 4-24®, to each jar. Concentrations of 0 ppm, 100 ppm, 250 ppm, 500 ppm, and 1000 ppm of TAGN were prepared in distilled water and thoroughly dissolved. Fifty ml of each concentration were then added to each of eight jars. After the media solidified, a small amount of yeast was added. Each group of eight jars containing a given concentration of TAGN was then divided into replications 1 through 8.

Specimens of Drosophila melanogaster, var. wild, were then anesthetized and separated by sex. Five males and five females were then placed in each jar. After the flies revived, the jars were placed in an environmental growth chamber set at 23°C and a photoperiod of 17 hours. The jars were monitored for several hours to determine if transfer procedures resulted in any deaths. Adult flies were removed from the jars on 29 June 1976, after a sufficient number of eggs had been laid. No flies had escaped during the 5 days. In order to determine the effects of TAGN on the development of the eggs, the number of pupae and/or larvae on the sides of the glass jars above the media were counted 5, 7, 13, and 19 days after flies were initially placed in the jars. At the termination of the experiment, 19 days after flies were placed in the jars, the adult flies in each jar were anesthetized and counted.

SECTION III

RESULTS

PART I - EFFECTS ON ADULT FLIES

The number of dead flies in each jar at 1, 2, 5, 7, 9, and 12 days after initiating the experiment are shown in Appendix I. No flies died as a result of treatment until the fifth day after initiating the experiment. An analysis of variance of the probability of a death by day was performed to determine if an interaction between sex and TAGN concentrations were significant at the 95 percent confidence level. It was determined that the sex of the flies had no influence on the death rate. As expected, sex alone was not found to be significant; however, death due to concentration of TAGN alone was significant at the 95 percent confidence level. During the progress of the experiment some flies escaped around the stoppers in some jars, which resulted in unequal sample sizes toward the end of the experiment. Because of this difference in sample size and the fact that the only response measured was whether or not the flies lived or died, a binomial distribution was used to analyze the daily results. All statistical analyses were performed at the 95 percent confidence level.

The probability of death due to TAGN in the growth medium is shown for the duration of the experiment in Figure 1. Table 1 shows the results of a multiple range test that divides means by statistical difference. On day 5 there was no difference in the number of dead flies between the control and 1000 ppm. The three highest concentrations, however, had significantly more dead flies per treatment than the 1000 ppm treatment although no difference was evident between the 3000 ppm and 4000 ppm treatments at that time. On days 7 and 9 the 1000 ppm treatment had caused more deaths than the control, and the 4000 ppm treatment was significantly different from the 3000 ppm treatment. There was no difference between 2000 ppm and 3000 ppm. By the end of the experiment on day 12 the number of dead flies in each treatment were significantly different from the rest, with the number of dead flies per treatment increasing with an increase in concentration of TAGN.

PART II - REPRODUCTIVE EFFECTS

The number of pupae/larvae in each jar at 5, 7, 13 and 19 days after initiating the experiment, and the number of flies in each jar at the termination of the experiment are shown in Appendix II. Data were subjected to an analysis of variance test to determine when differences between treatments became significant. It was determined that a difference at a 99 percent confidence level occurred between treatments beginning on day 5 and continued throughout the experiment.

The treatment means were then compared using a Multiple Range Test to show where differences occurred. Treatment means are shown in Figure 2.

On day 5 no significant difference was observed in numbers of pupae/larvae between any treatments that received TAGN. The control, however, had more pupae per jar than the other treatments. By day 7 the number of pupae in the 100 ppm treatment had become greater than in the higher concentrations, but not as high as the control treatment. On day 13 the number of pupae in all treatments were significantly different from each other, increasing in number with a decrease in concentration except that the control group had fewer pupae than the 100 ppm treatment. At the termination of the experiment on day 19, no significant differences were apparent between the control, 100 ppm, or 250 ppm treatments. However, the 500 ppm treatment had fewer pupae/larvae than any of those treatments and the 1000 ppm treatment had fewer than the 500 ppm treatment.

Adult flies were anesthetized and counted at the termination of the experiment on day 19. Treatments with TAGN at 500 ppm and 1000 ppm had the fewest flies per jar with no significant difference occurring between the two treatments. The 250 ppm concentration resulted in fewer flies per jar than the control, but the 100 ppm treatment was not found to be statistically different from the control treatment.

SECTION IV

DISCUSSION

TAGN appears to be relatively non-toxic to Drosophila as compared to data from the previous studies (Reference 5) on other compounds that are considered toxic to the species. In this experiment, concentrations of 250 ppm in the growth medium were required in order to affect the reproductive potential of Drosophila as measured by reduction of pupae/larvae production. Increasing the concentration to 1000 ppm TAGN in the medium resulted in almost all cessation of pupae and larvae production (Figure 2) as well as an approximate 50 percent death of adult flies after 12 days (Figure 1). Concentrations of 2000 ppm TAGN and above resulted in the death of almost all flies by the twelfth day after treatment. Comparatively, previous studies (Reference 5) have shown that Aldrin®, Dieldrin®, Heptachlor®, and Heptachlor Epoxide® required less than 0.15 ppm in the medium for a lethal dose. All these compounds are known for their toxic effect to insects such as Drosophila. TAGN is obviously much less toxic than these compounds.

Although this study alone has not provided sufficient data to determine the total potential environmental hazards associated with the use of TAGN, it does indicate that TAGN is relatively low in toxicity to the species studied. Since the material will necessarily be handled carefully because of its explosive classification, the toxicity of the compound does not appear to present a significant environmental or handling problem.

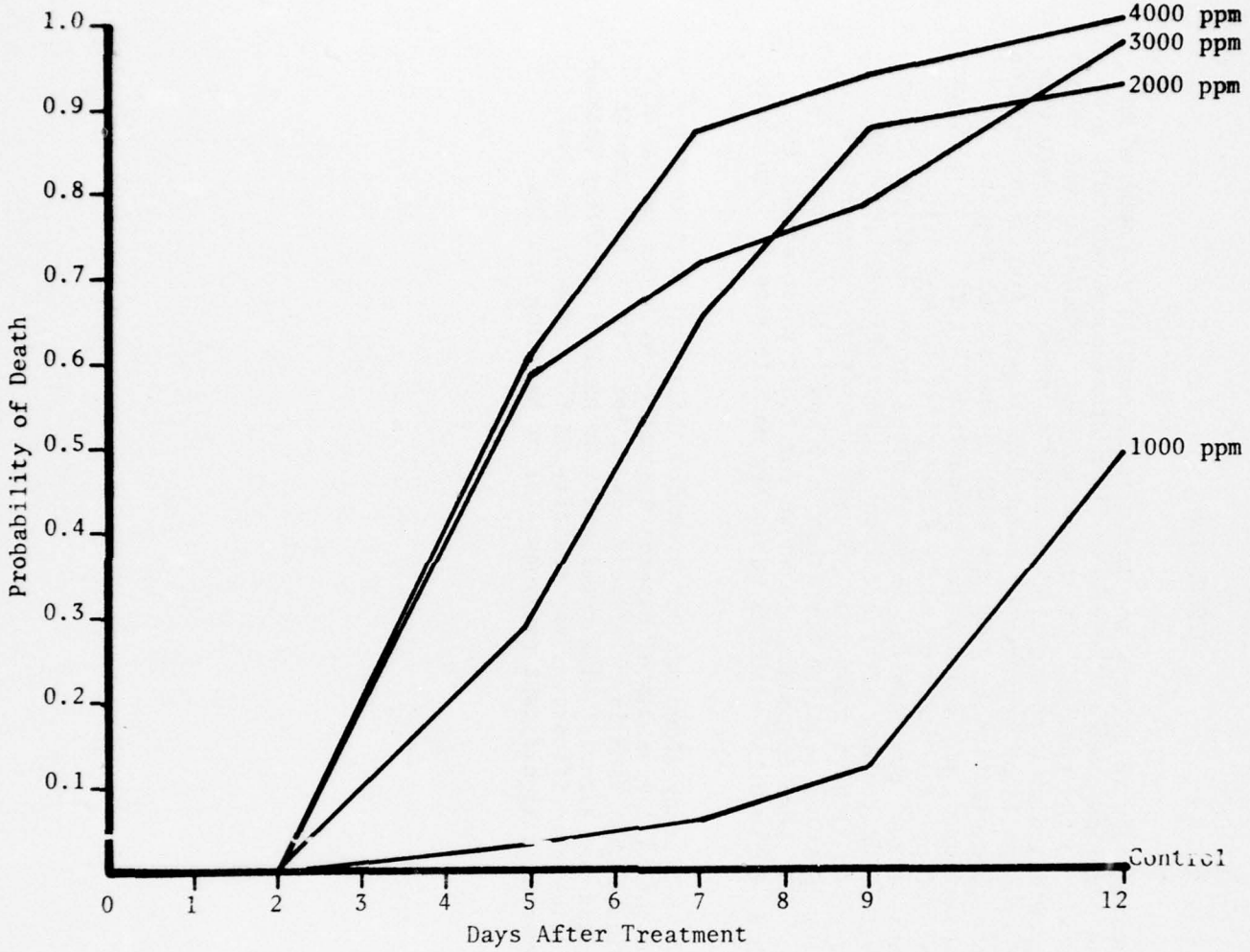


Figure 1. Probability of Death to Drosophila melanogaster, Var. Wild, Due to TAGN in Growth Medium

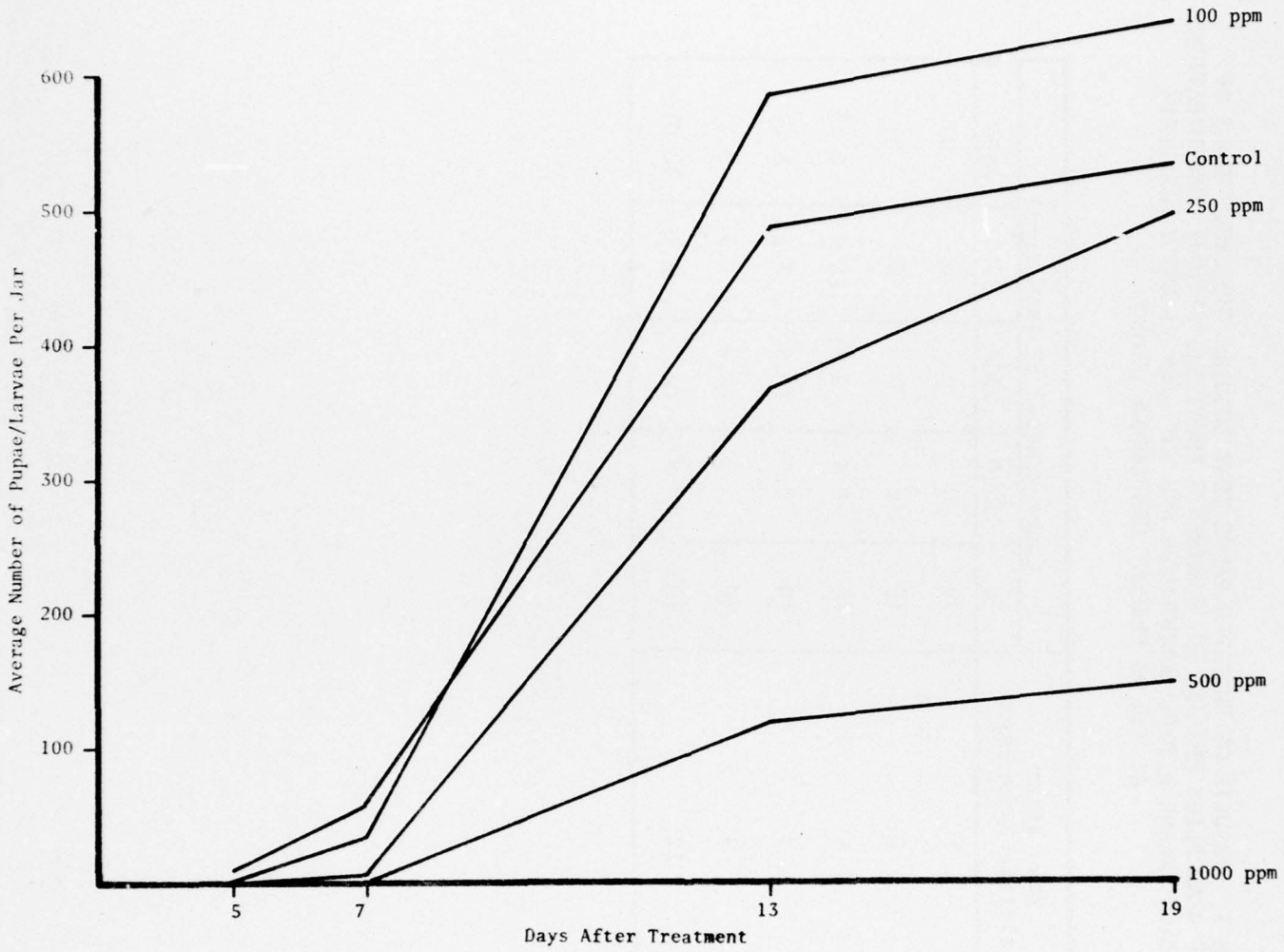


Figure 2. Effects of TAGN in Growth Medium on Production of Pupae/Larvae of Drosophila melanogaster, Var. Wild

TABLE 1. RESULTS OF MULTIPLE RANGE TEST SHOWING THE GROUP MEANS OF NUMBER OF DEAD FLIES PER JAR IN TREATMENTS RECEIVING VARIOUS CONCENTRATIONS OF TAGN (MEANS WITHIN PARENTHESES ARE NOT SIGNIFICANTLY DIFFERENT AT THE 95 PERCENT CONFIDENCE LEVEL)

Days after Initiating Experiment	Concentration of TAGN (ppm)				
	0	1000	2000	3000	4000
1	(0	0	0	0	0)
2	(0	0	0	0	0)
5	(0	0.4	2.9)	(5.9	6.1)
7	(0	0.6)	(6.6	7.1	8.8)
9	(0	1.2)	(8.8	7.9	9.2)
12	(0)	(4.9)	(9.2	9.7	10.0)

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APPENDIX A. NUMBER OF TOTAL/DEAD FLIES PER JAR

Treatment	Replication	Days after Initiating Experiment					
		1	2	5	7	9	12
Control	1	10/0	10/0	10/0	10/0	10/0	10/0
	2	10/0	10/0	10/0	10/0	10/0	10/0
	3	10/0	10/0	10/0	10/0	10/0	10/0
	4	10/0	10/0	10/0	10/0	10/0	10/0
	5	10/0	10/0	10/0	10/0	10/0	10/0
	6	10/0	10/0	10/0	10/0	10/0	10/0
	7	10/0	2/0	2/0	2/0	2/0	2/0
	8	10/0	10/0	10/0	10/0	10/0	10/0
1000 ppm	1	10/0	10/0	10/3	10/3	10/4	10/6
	2	10/0	10/0	10/0	10/0	10/0	10/0
	3	10/0	10/0	10/0	10/0	10/0	10/7
	4	10/0	10/0	10/0	10/0	10/0	10/6
	5	10/0	10/0	10/0	10/2	10/5	10/9
	6	10/0	10/0	10/0	10/0	10/0	10/1
	7	10/0	10/0	10/0	10/0	10/0	10/4
	8	10/0	10/0	10/0	10/0	10/1	10/6
2000 ppm	1	10/0	10/0	10/1	8/6	8/8	8/8
	2	10/0	10/0	10/0	10/2	10/10	10/10
	3	10/0	10/0	10/3	10/6	10/10	10/10
	4	10/0	10/0	10/0	10/0	10/0	10/4
	5	10/0	10/0	10/4	8/8	8/8	8/8
	6	* 9/0	9/0	9/3	9/9	9/9	9/9
	7	10/0	10/0	10/6	10/9	10/10	10/10
	8	10/0	10/0	10/6	10/9	10/10	10/10
3000 ppm	1	10/0	10/0	10/10	10/10	10/10	10/10
	2	10/0	10/0	10/10	10/10	10/10	10/10
	3	9/0	*	*	*	*	*
	4	10/0	10/0	10/0	10/0	10/2	10/10
	5	10/0	10/0	10/7	10/10	10/10	10/10
	6	10/0	10/0	10/0	10/1	10/3	10/8
	7	10/0	10/0	10/9	10/10	10/10	10/10
	8	10/0	10/0	10/5	10/9	10/10	10/10
4000 ppm	1	9/0	9/0	9/1	9/7	9/9	9/9
	2	10/0	10/0	10/10	10/10	10/10	10/10
	3	10/0	3/0	3/1	3/1	3/1	1/1
	4	10/0	10/0	10/4	10/10	10/10	10/10
	5	10/0	10/0	10/10	10/10	10/10	10/10
	6	10/0	10/0	10/8	10/10	10/10	10/10
	7	10/0	10/0	10/4	10/10	10/10	10/10
	8	10/0	10/0	10/8	10/10	10/10	10/10

* Numbers fewer than 10 indicate that some flies escaped.

APPENDIX B. NUMBER OF PUPAE/LARVAE AND ADULT FLIES PER JAR

Treatment	Replication	Days after Initiating Experiment				
					19	
		5	7	13	Pupae/Larvae	Adult Flies
Control	1	1	39	507	593	679
	2	3	40	478	494	591
	3	0	37	474	461	620
	4	20	87	479	514	537
	5	0	55	461	478	540
	6	11	63	529	548	555
	7	18	70	452	536	540
	8	20	63	525	633	645
100 ppm	1	0	29	476	609	480
	2	0	21	623	632	456
	3	1	32	661	698	684
	4	0	46	638	659	636
	5	1	51	566	603	501
	6	0	22	503	575	453
	7	0	7	607	653	583
	8	2	72	600	681	432
250 ppm	1	0	2	516	554	552
	2	0	3	165	239	231
	3	0	9	368	497	364
	4	0	1	449	486	367
	5	0	23	316	459	326
	6	0	7	361	550	353
	7	0	1	436	706	419
	8	0	0	336	483	303
500 ppm	1	0	1	89	103	102
	2	0	0	207	276	197
	3	0	0	93	127	109
	4	0	0	64	113	97
	5	0	0	65	86	70
	6	0	1	366	395	325
	7	0	0	62	74	56
	8	1	2	4	4	9
1000 ppm	1	0	0	0	0	4
	2	0	0	0	2	2
	3	0	0	0	0	2
	4	0	0	0	9	0
	5	0	0	0	0	0
	6	0	0	0	0	3
	7	0	0	0	0	1
	8	0	0	0	0	1

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